

## Detection of *Histoplasma capsulatum* in bats dropping by using polymerase chain reaction(PCR) at the first time in Iraq

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### Abstract

In the present we isolated the fungus *Histoplasma capsulatum* as a first time from bats dropping in AL-Qadisiyah province. Results show that the macro and micro characteristics referred that these characteristics was related to the fungus *Histoplasma capsulatum*, also this result was confirmed by PCR technique.

**Key words:** *Histoplasma capsulatum*, bats dropping, PCR

**Introduction:** *Histoplasma capsulatum* is a fungal pathogen that can result in a wide range of clinical presentations, from asymptomatic through fatal infection. It usually causes lung disease called Histoplasmosis or Darling's disease. It is called Darling's disease because it was found by Samuel Darling in histopathological specimens about a century ago. (Robert, 2001) *Histoplasma capsulatum* is a biologically interesting inhabitant of soil and mammalian hosts, a clinically significant cause of respiratory and systemic infection, and an excellent fungal model of dimorphic cell development and facultative intracellular pathogenesis. (Chang Ryan, 2007) *H. capsulatum* is unique in its dimorphism. Dimorphism allows *H. capsulatum* to infect mammals by going through three significant development stages depends on the temperature shift from 25 °C to 37 °C. (Bossche, 1993) In the moist soil that is rich in bird or bat guano at temperature about 25 °C, *H. capsulatum* exists in a filamentous mycelia form. However, when humans inhale *H. capsulatum* into their respiratory tracks, in order to replicate its DNA in the host at 37°C, the pathogen has to be able to convert its tissue from one form to another. In this case, *H. Capsulatum* changed from fungi to yeast when it's growing in the human bodies (Kauffman, 2007). *Histoplasma capsulatum* is found throughout the world. It is endemic in certain areas of the United States, particularly in states bordering the Ohio River valley and the lower Mississippi River. The humidity and acidity patterns of soil are associated with endemicity. Bird and bat droppings in soil promote growth of *Histoplasma*. Contact with such soil aerosolizes the micro conidia, which can infect humans. It is also common in caves in southern and East Africa. (Ryan and Ray, 2004). Regarding the presence of the fungus in Iraq is not registered according to available references we have and that the current study is the first in the country to record the presence of this fungus.

### Material & Methods:

**Culture media:** The following media were used for isolated and identification of fungus from samples (Beneke and Rogers, 1980):

\*Sabouraud's Dextrose Agar & chloramphenicol

\*Brain Heart Infusion Agar with cycloheximide & chloramphenicol \*Salvin's YP medium

**Collection of sample :** Samples of bats dropping were collected from bats nests where it was found. These samples were placed in sterile tins and transported to laboratory for culturing and testing.

**Isolation & Identification of fungus:** By using of method that reported by Lopez-Martinez & Castaron-Oliver (1995) was isolated the fungus from samples that collected. make suspension from the samples and cultured on the media that reported above. After the incubator period (at 25°C for two weeks and at 37°C for seven days) the fungus was identified as a *Histoplasma capsulatum* according to macro and micro features that reported by (Ellis, 1994; Larone, 1995; St-Germain & Summerbell, 1996; Frey et al., 2002)

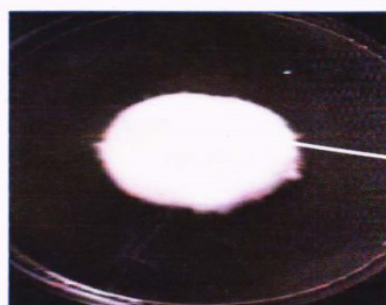
## PCR Technique:

**Extract DNA:** the DNA extracted from the isolates that selective on SDA medium, and Then followed the steps installed that supplied with the kit fitted from the company (Bioneer) to extract DNA, then blending part of it with dye Ethidium bromide on gel Agarose record in a concentration of 1.5% (100) ml buffer solution TEB Buffer concentration (1X) Molar according of the company (Bio Basic) for the purpose of purity way electric deportation.

**Prepare the reaction solution to a polymerase chain reactions PCR:** attended the reaction mixture (size 50 Micro liters) for a polymerase chain reactions following the steps installed and supplied with the kit AccuPower® PCR PerMix processed from the company (Bioneer) and by using of the *H. capsulatum*-specific primers HC-1 and HC-2 (HC-1: 5-GAGCCTCTGACCGGGAC-3; HC-2: 5-GCACGTCCCAC CCGGTCAG-3). (White et al., 1990)

## Results & Discussion:

Dimorphic fungi, such as *Histoplasma*, undergo a temperature induced transition between growth phases, which means that it has two distinct phenotypes. This dimorphism makes these organisms difficult to correctly identify using traditional microbiological methods. At ambient temperatures below 30°C in contaminated soils, *Histoplasma* grows in its mycelial form with the appearance of typical molds. Once the spores or microconidia have been inhaled, the organism then undergoes a metamorphosis to its yeast or parasitic form. It is at this stage that *Histoplasma* becomes virulent. At normal body temperatures, around 37°C, the organism looks and behaves as yeast with the typical budding off of cells. This phase change, induced by the elevated temperatures of the body, is required for virulence. (Benekli, 2004 ; Robert Brooks , 2011). Fig.(1) show colony with slow growing and cottony appearance , white in color . On other hand, Fig.(2) show septate hyphae with macroconidia, or tuberculate conidia, are 9 µm in diameter . Identification of the tuberculate macroconidia allows to diagnosis of *Histoplasma* (Bhatti, et al., 2004) Also, this result accordant with (Kauffman, 2003) that reported , the tuberculate conidia, are 8 to 15 µm in diameter and have distinctive projections on their surface. Fig (3) show moist, folded creamy colony at 37°C , that represented parasitic phase and fig(4) show , oval , unicellular budding yeast cells and buds arise at narrow neck . This results reported by (Beneke , 2004) whose reported that conversion to the yeast phase at 37°C on brain heart infusion agar are necessary criteria for identification of *Histoplasma capsulatum* . Many species of fungi other than *H. capsulatum* produce similar colony and sporulation characteristics. Some examples are *Blastomyces dermatitidis* *Chrysosporium* sp., and *Sepedonium* sp. . Therefore, additional testing is needed to definitively identify the organism. One method is to convert the fungus colony from the filamentous, mycelial phase to the yeast phase by subculture to highly enriched cysteine-containing media and incubation at 35° to 37°C. (Wheat, 1989).



Fig(1) White cottony colony on SDA (at 28°C)

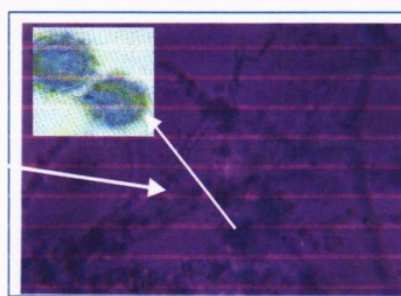
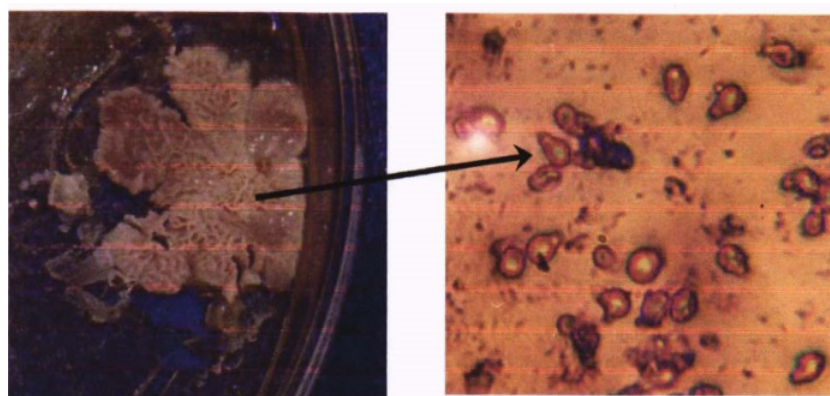


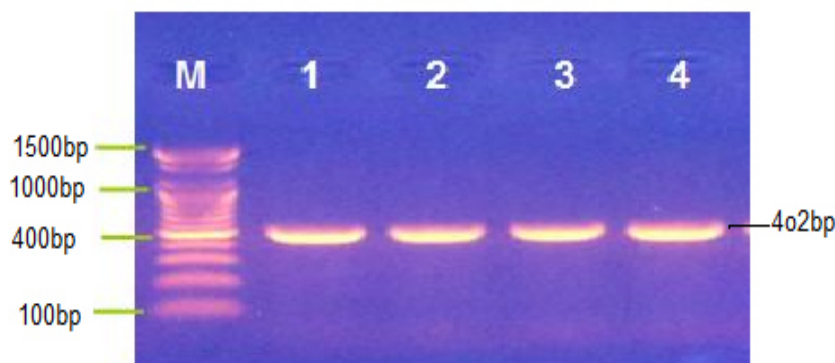
Fig.(2) Tuberculated macroconidia & small microconidia (640X)



Fig(3) Folded creamy colony on BHI (at 37C)

Fig.(4) yeast cells with capsule (640X)

DNA based PCR technology, which uses probes specific to these organisms, is a quicker, more accurate and cost-effective way of determining their presence or absence. Sequences that are found only in the genome of *Histoplasma* are targeted by the probes, allowing a precise, definitive result and do not require a viable organism for detection. (Hopfer, *et.al.*, 1993 ; Reid and Schafer, 1999). Fig.(5) shows, Polymerase chain reaction method (PCR) have been used to detection and identification of fungus, gave rise to a single 402bp PCR product. This result is accordant with Reid and Schafer (1999) that reported a polymerase chain reaction (PCR) method was developed that allows the direct detection of *H. capsulatum* in soil and gave to a single 400 bp PCR product diagnostic of *H. capsulatum*.



Fig(5): gel electrophoresis that the results of polymerase chain reaction to DNA gened diagnosis of *Histoplasma* sp. Where is the M: Marker ladder 100-1500 bp and samples of No. 1-4 represent positive samples for testing an output length of 402bp.

## Conclusion:

The data presented in this study for our knowledge is considered the first screening data regarding the incidence of *Histoplasma* in Iraq and the high incidence was observed in bats dropping samples. PCR technique has an important role of diagnosis of this fungus and this assay that correctly identified *H. capsulatum* from among a variety of fungi grown in the laboratory appears promising.

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